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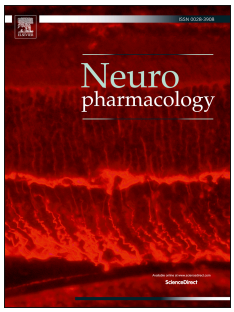

$GABA_B$ receptors and pain

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$GABA_B$ receptors and pain

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Abstract

Over the past three decades the research on $GABA_B$ receptor biology and pharmacology in pain processing has been a fascinating experience. Norman Bowery's fundamental discovery of the existence of the $GABA_B$ receptor has led the way to the definition of $GABA_B$ molecular mechanisms; patterns of receptor expression in the peripheral and central nervous system; $GABA_B$ modulatory functions within the pain pathways. We are now harnessing this acquired knowledge to develop innovative approaches to the therapeutic management of chronic pain through allosteric modulation of the $GABA_B$. Norman's legacy would be ultimately fulfilled by the development of novel analgesics that activate the GABAB receptor.

Key words: $GABA_B$ receptor, nociception, inflammatory pain, neuropathic pain

Introduction

The $GABA_B$ receptor agonist (±)baclofen (β p-chlorophenyl-GABA)(Lioresal) is the drug of choice for spasticity in multiple sclerosis and spinal injury and it is also used as analgesic for chronic pain associated with spinal cord injury and trigeminal neuralgia. Baclofen's side effects such as sedation and motor impairment limit its systemic use, and intrathecal delivery is an alternative route of administration which also overcomes baclofen's poor brain penetration (Bowery, 2006).

In the nineties we knew that (-)baclofen was the stereo-selective ligand for the $GABA_B$ receptor which had been recognised by Norman Bowery as a receptor for GABA, which was different from the $GABA_A$ receptor (Hill and Bowery 1981). $GABA_B$ receptor antagonists were also synthesised and made available by the CIBA-Geigy laboratories (Olpe et al., 1990). However, it took a number of years for the gene encoding the metabotropic $GABA_B$ receptor to be cloned by Klemens Kaupmann and for $GABA_{B1}$ and $GABA_{B2}$ heterodimers to be identified as critical units for functional expression (Kaupmann et al., 1997; White et al., 1998). $GABA_B$ knock out mice were generated by the Novartis team 5 years after receptor cloning with the aim to establish the role of $GABA_B$ receptor in in vivo models of epilepsy, memory and pain (Schuler et al., 2001).

In the early 90's it became evident that the systemic administration of (±)baclofen resulted in analgesic and anti-hyperalgesic effects in experimental models of acute and chronic pain at doses which were significantly lower than those required for muscle-relaxation. Baclofen exerted antinociceptive effects when administered directly in the CNS by intrathecal and intracerebroventricular injections (Sawynok and LaBella, 1982; Wilson and Yaksh, 1978). Consistent with a central site of action for baclofen, $GABA_B$ receptor antagonists injected intrathecally were able to prevent the analgesic effect of systemic baclofen (Malcangio et al., 1991). Indeed, autoradiography binding studies demonstrated that $GABA_B$ receptors in the

spinal cord were localised mainly in the superficial laminae of the dorsal horns where they were found on both primary afferent fibre terminals and intrinsic neurons (Malcangio et al., 1993; Price et al., 1987; Price et al., 1984). The expression of $GABA_B$ receptors in the dorsal horn showed plasticity and underwent up- or down-regulation after systemic treatment with receptor antagonists or baclofen (Malcangio et al., 1993). $GABA_B$ receptor plasticity explained the tolerance to the analgesic effect of baclofen that developed after prolonged treatments and it limited the chronic use of the drug (Malcangio et al., 1993). At the same time it was clear that baclofen-induced analgesia was effected through a novel pathway, independent from the opioid system as it was not reversed by naloxone. Equally, the pathway was distinct from $GABA_A$ receptor activation as the effect of baclofen was not blocked by bicuculline.

$GABA_B$ receptors

The cloning of the two $GABA_B$ receptor subunits demonstrated that $GABA_B$ receptors are metabotropic members of the seven transmembrane G-Protein coupled receptors superfamily, which belong to the same class as metabotropic glutamate receptors (mGluRs), calcium-sensing receptors and receptors for umani and sweet taste. $GABA_B$ receptor activation is associated with opening of inwardly rectifying potassium channels, inhibition of calcium channels and activation of adenylyl cyclase. $GABA_B$ receptors have a heterodimeric structure and complete receptor function requires the assembly of $GABA_{B1}$ and $GABA_{B2}$ subunits, which are linked by protein/protein interactions between their intracellular C-terminal domains. The GABA binding site is located on the extracellular domain of the $GABA_{B1}$ subunit, whilst the $GABA_{B2}$ subunit interacts with the G proteins and is essential for functional expression of the receptor. $GABA_B$ receptor subunits are found both centrally and peripherally including the thalamus, brain stem nuclei and spinal cord.

Acute and chronic pain

Pain is a subjective experience associated with actual or potential tissue damage. It is a sensation in a part of our body that is also unpleasant, and therefore an emotional experience. Pain can be acute or chronic: acute pain is a warning system essential for survival and well-being whilst chronic pain does not serve any useful protective mechanism for the organism, outlasts the noxious stimulus and is poorly controlled by current analgesics (Woolf, 2010). The pain experience is mediated by specialised primary afferent fibres, namely the nociceptors, which detect noxious stimuli in the periphery and transmit electrical impulses centrally to the spinal cord on their way to the brain where pain is perceived**.** Interestingly, the underlying organization and strengthening of nociceptive circuitry in the dorsal horn in adulthood can be altered by sensory inputs in early life (Fitzgerald, 2005) and during early development GABA can induce depolarization due to reduced neuronal ability to extrude chloride ions (Baccei, 2016).

There are profound differences between acute and chronic pain when dramatic changes occur in peripheral nociceptors and central nociceptive pathways and the pain system is sensitised thereby leading to exaggerated responses to noxious stimuli (hyperalgesia) and responses to non-noxious stimuli (allodynia) (Sandkühler, 2009). Tissue injury results in inflammatory pain in diseases such as rheumatoid arthritis (Üçeyler et al., 2009). Neuropathic pain results from lesions to the peripheral nervous system caused by mechanical trauma, metabolic diseases such as diabetes or neurotoxic chemicals such as chemotherapeutic agents. In chronic pain states the increased nociceptive input from the periphery triggers the physiological plasticity and long lasting transcriptional and post-translational changes in the CNS defined as *central sensitization* (Kuner, 2010). Glial cells in the spinal cord, such as microglia and astrocytes, also contribute to central sensitization (Malcangio, 2016; McMahon and Malcangio, 2009) and cortical and sub-cortical structures modulate pain (Eippert et al., 2009). Under neuropathic pain conditions, loss of function of inhibitory neurons at the level of the spinal cord contributes to the increased excitation and in chronic inflammatory pain GABAergic inhibition is decreased in the spinal cord (Takazawa et al., 2017; Zeilhofer et al., 2012).

GABA and $GABA_B$ receptors in pain

GABA is expressed by inhibitory interneurons in the laminae I-III of the dorsal horns which constitute a different neuronal population from excitatory interneurons expressing glutamate (Polgar et al., 2003; Polgár et al., 2013; Zeilhofer et al., 2012). GABAergic islet cells that contain parvalbumin are innervated by myelinated primary afferent fibres which receive axoaxonic synapses from GABAergic cells. Such GABA-mediated pre-synaptic inhibition is likely to regulate myelinated fibres activity following non-noxious mechanical stimulation in the periphery. GABAergic interneurons which also contain neuropeptide Y innervate projection neurons expressing NK_1 receptor for Substance P (SP) and those which also contain nNOS innervate giant cells in lamina I. As NK_1 projection neurons are innervated by the nociceptive C fibres, these GABAergic synapses are likely to regulate activity in response to nociceptive inputs from the periphery.

GABA modulates nociceptive transmission at the level of the dorsal horn through activation of both $GABA_A$ and $GABA_B$ receptors that are located on primary afferent terminals as well as dorsal horn neurones, including lamina I spinothalamic projection neurons (Calver et al., 2000).

In the spinal cord $GABA_B$ receptors regulate the activity of both peptidergic primary afferent terminals and dorsal horn neurons. Indeed, baclofen application to the dorsal horns isolated in vitro inhibits the release of SP evoked by activation of primary afferent fibres (Malcangio and Bowery, 1993) as well as the internalization of the NK_1 receptors on projection neurons (Marvizon et al., 1999). Baclofen inhibits C fibre-evoked activity of convergent/multireceptive neurons in anaesthetised rats (Dickenson et al., 1985). Furthermore, baclofen inhibits c-fos expression in dorsal horn neurons after exogenous SP application (Riley et al., 2001). Notably, the effect of baclofen on NK_1 receptor internalization are significant after intrathecal rather than systemic administration which results, as discussed above, in a predominant

muscle relaxant effect (Riley et al., 2001). $GABA_B$ receptors are localized on the terminals of peptidergic primary afferents fibers (Malcangio et al., 1993; Price et al., 1984). At presynaptic sites the $GABA_B$ receptor activation leads to inhibition of high voltage-gated Ca^{2+} channel activity and inhibits glutamate as well as SP and CGRP release (Bowery, 2006; Malcangio and Bowery 1995; Malcangio and Bowery, 1993; Marvizon et al., 1999). The decrease of dorsal horn neuron excitability (Kangrga et al., 1991), and the regulation of intrinsic neuronal properties (Derjean et al., 2003) suggest an additional postsynaptic site for the baclofen action on pain.

As the effects of baclofen on primary afferent activity are not reproduced by $GABA_A$ receptor agonists like muscimol and isoguvacine, this ionotropic receptor may play minimal role in the modulation of primary afferent activity. However, it is important to note that post-synaptic $GABA_A$ receptors remain critical for mediating GABA-induced analgesia and indeed some benzodiazepine that are GABA mimetic drugs at the $GABA_A$ receptors exert analgesic effects. In the case of systemic diazepam, the site of action appears to be the spinal cord where $GABA_A$ receptor subunits $\alpha 2$ and $\alpha 3$ are responsible for analgesic effects (Knabl et al., 2008; Knabl et al., 2009).

$GABA_{B1}$ knock out mice which lack GABA binding sites and functional $GABA_B$ receptors display reduced sensitivity to hot and mechanical nociceptive stimulation and confirm the existence of a $GABA_B$-mediated tone of pain and the possibility that $GABA_B$ modulators would act synergistically with endogenous GABA.

$GABA_B$ and inflammatory pain

Under inflammatory pain conditions such as in rat models of monoarthritic inflammatory pain, GABA is up-regulated in dorsal horn superficial laminae (Castro-Lopes et al., 1992) and down-regulated in the ventrobasal thalamus (Zhang et al., 2017) and animals are less sensitive to the analgesic effect of systemic baclofen (Castro et al., 1999; Malcangio and Bowery, 1994). Recent evidence indicates that GABA-mediated inhibitory activity is

decreased in the dorsal horn after peripheral inflammation and contributes to reduced inhibition under persistent pain conditions (Takazawa et al., 2017).

Whilst selective deletion of peripheral $GABA_{B1}$ receptor subunit in sensory neurons indicated no major role in inflammatory pain (Gangadharan et al., 2009), both $GABA_B$ receptor activation in the spinal cord and ventrolateral thalamus contribute to the anti-nociceptive effect of baclofen (Buritova et al., 1996; Potes et al., 2006). Recently, GABA expression has been identified in sensory neurons which also express the $GABA_{B1}$ receptor subunit in close juxtaposition with $TRPV_1$ receptors. A fascinating possibility is that GABA via activation of $GABA_{B1}$ autoreceptor can attenuate sensitization of $TRPV_1$ receptor in nociceptors (Hanack et al., 2015). A strategy which has yielded significant success evolved around the development of positive allosteric modulators, or PAMs. PAMs provide an opportunity for selectivity as these molecules become operative only in the presence of the endogenous agonist, when they display the positive enhancing effect on receptor activation. The rationale herein would be to positive modulate $GABA_B$ receptor activity only at synapses where GABA is released. Of importance, PAMs can enhance activity of orthosteric agonists like baclofen. Congruently, ADX71441, a compound developed very recently, showed limited muscle relaxant activity but significant anti-nociceptive activity in the inflammatory phase of the monoiodoacetate model of osteoarthritis (Kalinichev et al., 2017).

$GABA_B$ and neuropathic pain

In neuropathic pain conditions, GABAergic inhibitory control at the level of the spinal cord is significantly reduced and contributes to increased excitation and central sensitization of pain transmission. Such loss of inhibition is the result of several possible mechanisms including death of inhibitory neurons (Castro-Lopes et al., 1992; Ibuki et al., 1996; Moore et al., 2002; Yowtak et al., 2013) but see (Polgár and Todd, 2008), diminished neuronal activity and decreased release of GABA (Leitner et al., 2013; Lever et al., 2003; Schoffnegger et al., 2006). Despite the reduced endogenous GABAergic tone, baclofen exerts anti-nociceptive effects in neuropathic animals and intraspinal transplantation of cortical precursors of

GABAergic interneurons from the medial ganglionic eminence (MGE approach) which can release GABA, can reverse neuropathic allodynia (Bráz et al., 2015). Notably, MGE approach is effective in chemotherapy-induced pain models in which GABA levels are not altered (Bráz et al., 2015). In addition, still in models of chemotherapy-induced neuropathy, significant up-regulation of $GABA_B$ receptors in sensory neurons mediates the sustained analgesic effect of the opioid oxycodone (Thibault et al., 2014).

Neuropathic animals display increased sensitivity to the anti-nociceptive effect of baclofen and whilst the number and affinity of $GABA_B$ binding sites in the dorsal horn are not altered (Smith et al.,1994; (Zemoura et al., 2016), $GABA_B$ receptors subunits (B1 and B2) are both down-regulated in sensory neurons after nerve injury (Engle et al., 2012) and $GABA_{B1}$ down-regulation in nociceptive neurons is a likely contributor to reduced GABA-mediated inhibition of nociceptive input in the dorsal horn. Allosteric modulators of $GABA_B$ receptors such as rac-BHFF, have not shown efficacy in neuropathic mice and suggest a limited role for that $GABA_B$-mediated mechanisms under this condition (Zemoura et al., 2016)

On a more positive note, baclofen has shown anti-hyperlagesic effects in cancer induced bone pain (CIBP) which is poorly managed with currently available analgesics including opioids. $GABA_B$ receptors undergo down regulation in the dorsal horn of CIBP rats where they are expressed mainly by neurons. Interestingly, prolonged treatment with baclofen restored receptor expression (Zhou et al., 2017). Whether $GABA_B$ allosteric modulators are effective analgesic in CIBP remains to be explored.

Conclusion

In conclusion, over the past three decades the research on GABA biology and pharmacology has been a fascinating voyage. From the intuition of Norman Bowery on the existence of a second receptor for GABA to the definition of $GABA_B$ molecular mechanisms, patterns of expression and potent modulatory functions within the CNS, we are now harnessing this science for innovative approaches to the therapeutic management of pain. In this review I have brought together this knowledge which has stemmed from Bowery's discovery with the

excitement and awareness that his legacy would be fulfilled by the development of novel analgesics able to activate the $GABA_B$ receptor.

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Over the past three decades the research on $GABA_B$ receptor biology and pharmacology in pain processing has been a fascinating experience. Norman Bowery's fundamental discovery of the existence of the $GABA_B$ receptor remains essential for the discovery of novel analgesics that activate the $GABA_B$ receptor.